

Transformation of *Bacillus subtilis* (simple)

- inoculate 10 ml MNGE to $OD_{600} = 0,1$ (or better 1/100) from overnight culture
- let grow to $OD_{600} = 1.1-1.3$ at 37°C with agitation (at least 200 rpm!)
- use 400 µl cells for transformation (in glas test-tube, not eppendorf!):
 - o add DNA (2 µg linearized plasmid or 100 µl crude-prep genomic DNA)
 - o let grow for 1 h
 - o add 100 µl Expression Mix (may need to pre-induce: for MLS use Ery 0,025 µg/ml final and for Cm use Cm 0,125 µg/ml final)
 - o let grow for 1 h
 - o plate on selective media

10 X MN-Medium (1 L):

136 g K_2HPO_4 (x 3 H_2O)
60 g KH_2PO_4
10 g Na-citrat (x 2 H_2O)

MNGE-Medium:

9,2 ml 1 x MN-Medium (920 µl 10x MN + 8,28 ml sterile water)
1 ml Glucose (20%)
50 µl K-Glutamate (40%)
50 µl Fe[III]- ammonium-citrate (2,2 mg/ml)
100 µl Tryptophan (5 mg/ml)
30 µl $MgSO_4$ (1M)
(100 µl threonine (5 mg/ml) for strains carrying an insertion in *thrC*)

Expression Mix:

500 µl yeast extract (5%)
250 µl casamino-acids (CAA) (10%)
250 µl H_2O
50 µl Tryptophan (5 mg/ml)